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Short communication

New analgesic and antiinflammatory agents 4(1H)-pyridinone derivatives

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Abstract

A series of 1,2,5-trisubstituted 4(1H)-pyridinone derivatives (**7**–**14**) were synthesised by using 4-pyrone derivatives with primary amines in ethanol. The structures of the synthesised compounds were confirmed by analytical and spectral data (UV, IR and ^{1}H-NMR and microanalysis). Analgesic and antiinflammatory activities of the synthesised compounds were investigated by acetic acid-induced writhing syndrome and carrageenan rat paw edema tests. All of the test compounds exhibited higher analgesic activities than acetyl salicylic acid and showed higher antiinflammatory activities than indomethacin. The anti-inflammatory activity and gastric ulceration potential of the compounds were tested using indomethacin as reference drug.

Keywords: 4(1H)-pyridinones; Analgesic agents; Antiinflammatory agents; Synthesis; Kojic acid derivatives

1. Introduction

A large number compounds with a 4(1H)-pyridinone structure have been synthesised for biological screening and variety of interesting pharmacological properties were observed [1–3]. The most important activities regarding their analgesic and anti-inflammatory actions belong to their iron chelating properties. Moreover, several 4(1H)-pyridinone compounds have shown analgesic and anti-inflammatory activities [1,4]. The most prevalent side effects of the use of non-steroid antiinflammatory drugs are the occurrence of gastrointestinal damage with gastric upset and irritation being the major problems. Therefore, developing new agents with or without minimal side effects is, at present, a diffuse research area. Recently, in an effort to synthesise analgesic and antiinflammatory drugs with minimal gastrointestinal side effects, 4(1H)-pyridinones have emerged as a promising group [5,6].

The reaction of primary amines with 4(1H)-pyrones to form N-substituted-4(1H)-pyridinones has been known for more than 90 years [7]. It has been utilised for the preparation of a variety of these pyridinones [7–9]; despite the fact that this reaction has somewhat limited scope, being reliable only for small alkyl and aryl amines. It is well known that 4(1H)-pyridinone derivatives have shown various pharmacological effects such as antibacterial [10,2], antifungal [11], antimalarial [12,13], cardiotonic agents [14], antihypertensive [15], antineoplastic [16–19], antiinflammatory [1], analgesic [20–23], and treatment of Parkinson's disease [3,24]. On the other hand, 3-hydroxy-2-methyl-4(1H)-pyridinone derivatives are of considerable interest because they may be used as orally active iron (III) chelators in the treatment of iron overload and acute iron poisoning [25–29]. Hydroxypyridinones are undergoing development for the treatment of Thalassemia disease [30,31].

This consideration led us to synthesise a series of 4-pyridinone derivatives substituted with several cyclic amines such as piperidine, pyridine, pyrrolidine and morpholine by linking through an ethylene moiety (Fig. 1). The existence of the relationship between inhibition of prostaglandin biosynthesis and analgesic or antiinflammatory activities was investigated in the case of 4-pyridinone derivatives in comparison to acetyl salicylic acid and indomethacin.

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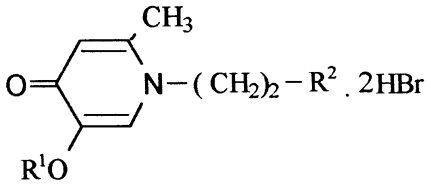


R¹ : H, -CH_2-C_6H_5

R² :

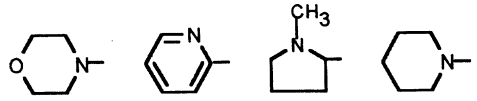


Fig. 1. 1,2,5-Trisubstituted 4(1H)-pyridinone·2HBr derivatives.

2. Chemistry

The synthesised compounds [**7**–**14**] were obtained by reacting primary amines such as 1-(2-aminoethyl)piperidine, 2-(2-aminoethyl)pyridine, 2(2-aminoethyl)-l-methylpyrrolidine and 4-(2-aminoethyl)morpholine with 4-pyrone derivatives in ethanol. The desired substitited-4(1H)-pyridinones were catechol derivatives by cleavage with BBr_3 in dichloromethane. Susceptibility of the free bases to air oxidation necessitated preparation of the corresponding hydrobromide salts for storage and biological testing.

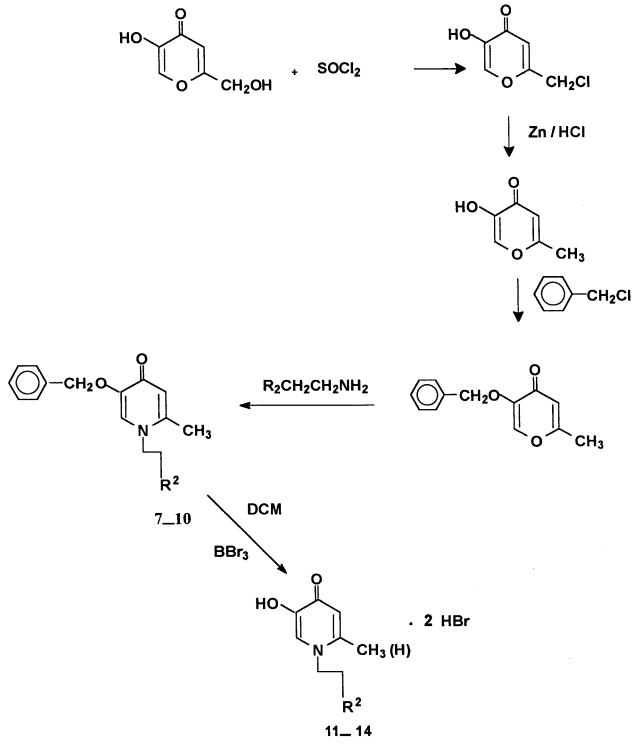
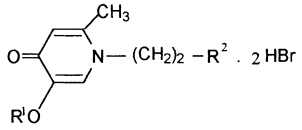


Fig. 2. Synthesis of 1,2,5-trisubstituted-4(1H)-pyridinone·2HBr derivatives.

Since a convenient route to 4-pyridones is the condensation of 4-pyrones with primary amines, commercially available kojic acid was selected as starting materials for the synthesis of the series of 4-pyridone analogs (Fig. 2). Condensation of the benzyl ether of the starting material with primary amines affords the desired pyridones. The hydroxymethyl group required conversion to benzyl kojic acid as a protection of phenolic function. Formulas, melting points, % yields of compounds are shown in Table 1.

The basic structures of these compounds were confirmed by IR and ^{1}H-NMR spectral data Table 2. The reaction products were assigned structures that were in accord with their spectroscopic and chemical behaviour. Thus all these compounds showed a strong band at 1632 cm^{-1} in their IR spectra assignable to a C=O group, and at 1234 cm^{-1} which is characteristic of a C–O–C band. The ethylene group protons in the ^{1}H-NMR spectra of all compounds appeared as a triplet at 4.70–3.50 ppm for –N–CH_2–, a triplet signal at 3.90–2.90

Table 1
Structures and chemical data of *N*-sustituted-2-methyl-5-hydroxy-4(1H)-pyridinones·2HBr **7**–**14** derivatives



Compound No.	R^1	R^2	Yield [%][a]	Melting Point[b] [°C]	Molecular Formula	Analysis
7	-$CH_2C_6H_5$		85	Liquid	$C_{20}H_{26}N_2O_2$	C,H,N
8	-$CH_2C_6H_5$		78	Liquid	$C_{20}H_{20}N_2O_2$	C,H,N
9	-$CH_2C_6H_5$		73	Liquid	$C_{20}H_{26}N_2O_2$	C,H,N
10	-$CH_2C_6H_5$		91	Liquid	$C_{19}H_{24}N_2O_3$	C,H,N
11	H		72	238-9	$C_{13}H_{20}N_2O_2.2HBr$	C,H,N
12	H		55	280-1	$C_{13}H_{14}N_2O_2.2HBr$	C,H,N
13	H		50	230 dec.	$C_{13}H_{20}N_2O_2.2HBr$	C,H,N
14	H		68	235 dec.	$C_{12}H_{18}N_2O_3.2HBr$	C,H,N

[a] Yields are those of the products obtained from the first ctystallization (ethanol–ether).

[b] Melting points were determined on a Thomas Hoover apparatus and are uncorrected.

[c] C,H,N were performed by Butterworth Co., UK.

Table 2
Spectral data of N-substituted-2-methyl-5-benzyloxy-4(1H)-pyridinones (**7**–**10**) and N-substituted 2-methyl-5-hydroxy-4(1H)-pyridinones·2HBr (**11**–**14**) derivatives

Compound number	1R (cm^{-1}) [a]		^{1}H-NMR (ppm) [b,c]					
	C=O	C–O–C/OH*	CH_3 [s]	N–CH_2 [t]	CH_2 [t]	O–CH_2[s]	$H^{3,2}$ [s,d]	H^6 [s]
7	1634	1229	2.50	3.50	3.10	5.35	6.30	7.80
8	1631	1233	2.45	4.05	2.90	4.90	6.10	6.80
9	1631	1229	2.65	3.50	3.00	5.15	6.30	7.00
10	1632	1234	2.65	3.95	3.10	5.25	6.30	6.90
11	1614	3500–3400	2.50	4.65	3.15	–	7.00	7.90
12	1634	3200–3100	2.40	4.55	3.35	–	6.90	8.00
13	1636	3400–3100	2.65	3.75	3.20	–	7.15	8.15
14	1616	3500–3200	2.55	4.70	3.70	–	6.90	7.95

s, Singlet; d, dublet; t, triplet.
[a] IR spectra were determined on a Perkin–Elmer FTIR Spectrometer 1720 X in KBr pellets.
[b] ^{1}H-NMR spectra were charted on a Bruker AC 80 MHz FT.
[c] Bruker AC 200 MHz FT and compounds were solved in $CHCl_3$-d_6 (**7a**–**14a**) and DMSO-d_6 (**7**–**14**).

ppm for –N–CH_2–CH_2–. Characteristic doublets peak of pyridones were observed at aromatic field.

3. Pharmacology

The experiments were carried out on male albino-Swiss mice (body weight of 20±2 g) and purchased (local breed) from the University of Hacettepe Animal House and maintained at a temperature between 20 and 23 °C. The animals were housed in groups of eight, with food and water ad libitum and allowed to get accustomed to their environment for at least 2 days before test started. Motor coordination was measured according to the method of Gross et al. [32] Mice were placed for 2 min on the rod rotating with the speed of 4 rpm. The effects were evaluated 15, 30, 45, 60 and 75 min after the administration of the investigated compounds. Animal experiments have been practised under the supervision of Veterinary Faculty of Ankara University, which is responsible for the observation of the rules of guidelines of animal experiments.

3.1. Analgesic activity

Pain reactivity was measured by the 'writhing syndrome' test of Koster et al. [33]. The test was performed in mice by i.p. injection of a 3% solution of acetic acid 60 min after the administration of compounds. Each compound was administrated intraperitoneally (i.p.) as a solution in Tween 80 of 100, 50 and 25 mg kg^{-1}. Control groups received an equal volume of vehicle. Each experimental group consisted of eight animals. Animals were placed in glass cages 5 min after acetic acid injection and number of 'writhes' induced in each mouse was observed for a 10 min period, starting 5 min after injection of acetic acid. Acetylsalicylic acid (ASA) was used as a reference analgesic drug and administered according to the test protocol (100 mg kg^{-1}).

The analgesic activity was expressed in terms of % inhibition;

$$\%\ \text{Analgesic Activity} = n - n'/n100$$

n is the mean number of writhes of control group, and n' is the mean number of writhes of test group.

3.2. Antiinflammatory activity

Carrageenan induced mouse paw edema (CPE) was measured using Peacock Thickness gauge (0.01–10 mm), eight mice per group were used. Sixty minutes after i.p. administration of the compounds (100 mg kg^{-1}), 2% carrageenan (0.01 mL) was injected subcutaneously into the plantar surface of the right hind paw. Two hours later, the volume of the edema was measured. Indomethacin [100 mg/kg i.p. injection] was used as a positive control [34].

3.3. Gastric ulceration studies

Active compounds were subjected to this experimental process. Animals were sacrified after last measurement application of each test compounds, under ether anesthesia and stomachs were removed, opened through the greater curvature, washed and fixed in 5% formalin solution. The stomachs were then examined for lesions under a dissecting microscope.

3.4. Statistical values

Student t test and analysis of variance: ANOVA, two factors [pharmacologic calculation system version 4.1] were employed.

4. Results and discussion

Formulas, melting points, % yields of the compounds are shown in Table 1. The synthesized compounds (**7a**–**14a**) were obtained by reacting primary amines with 4-pyrone derivatives in ethanol. The desired substituted-4(1H)-pyridinones were cathechol derivatives by cleavage with BBr_3 in dichloromethane. Susceptibility of the free bases to air oxidation necessitated preparation of the corresponding hydrobromide salts for storage and biological studies.

Substituted 4(1H)-pyridinone derivatives were screened for their analgesic and antiinflammatory activities. A modified Koster Test for screening analgesic activity was employed, carrageenan-induced mousse paw edema for screening antiinflammatory activity was measured using Peacock Dial Thickness Gauge. All tested compounds had no neurotoxic properties as they did not affect the motor coordination in the rotarod test.

In the present study, acetic acid 3& (300 mg/kg) was used to induce abdominal contractions (writhing) in the mice. Koster et al. [33] used very low dose acetic acid (60 mg/kg) for evaluating writhing response in mice. In our preliminary studies, we did not observe marked and acceptable writhing responses by this low dose of acetic acid. In some previous studies, acetic acid 3% (300 mg kg^{-1}) has also been used for evaluating writhing responses in mice [33,34]. Using different mice species may be responsible for the discrepancy. As for the analgesic activity, all the compounds were shown to be equally potent in comparison with aspirin. The degree of protection ranged from 51 to 88% at a dose of 100 mg kg^{-1}. The most active compounds were **11**, **13** and **14** which displayed a significant analgesic effect also at dose of 50 mg kg^{-1} (73.22, 75.94 and 68%, respectively) and 25 mg kg^{-1} (58.65, 53.87 and 49.67%, respectively). Compound **13** is the more promising one and slightly more potent than the others. Antiinflammatory activities of all the synthesised compounds were screened using the carrageenan hind-paw edema test with indomethacin as standard. These results are disclosed in Fig. 3. The edema inhibition of all compounds was significant when compared with the inhibition obtained by indomethacin. In Fig. 3 the activity of compound **13** is shown significantly high and potent in comparison with indomethacin. The obtained results indicate that the investigated 4(1H)-pyridinone derivatives have a profile of action corresponding to new analgesic and anti-inflammatory agents.

The stomachs of the animals were also examined for gastric ulceration and in spite of the high gastric ulcer incidence with the reference compound indomethacin the synthesised active compounds **11**, **13**, **14** (25 and 50 mg kg^{-1} doses, 0/6 ulcer score) were generally found safe from the point of view of ulcer induction.

Therefore, we can conclude that the 1-pyrrolidinoethyl-2-methyl-5-hydroxy-4(1H)-pyridinone·2HBr (compound **13**) is the most active of the series as analgesic and anti-inflammatory compound and furthermore, the ulcerogenic activity of the compound **13** was lower than that of the reference compound and showing almost no ulcerogenic activity.

5. Experimental protocols

Melting points (Table 1) were determined in open glass capillaries on a Thomas-Hoover (Philadelphia, USA) apparatus and are uncorrected. The infrared spectra (Table 2) were recorded on a Perkin–Elmer (Table 2) FTIR 1720 X IR (Beaconsfield, UK) spectrophotometer using samples in potassium bromide disks. ^{1}H-NMR spectra were measured on a Perkin–Elmer R.32 90 MHz and Bruker AC 200 MHz FT NMR (Karlsruhe, Germany) using tetramethylsilane as internal standard and dimethyl-d_6 sulfoxide as solvent. Chemical shift values are reported as δ (ppm) values. Mass spectra were obtained from V6 16F mass spectrometer with V6 data system 2000. Analyses indicated by elemental symbols were within $\pm$0.4% of the theoretical values and were performed by Butterworth, UK. The purity of the compounds was determined by TLC on silica gel HF 254 (Merk) (Chloroform–Methanol 95:5). All chemicals were obtained from Aldrich Chemical Co. (Steinheim, Germany).

5.1. 2-Chloromethyl-5-hydroxy-4-pyrone (*1*)

Kojic acid (142 g, 1 mol) was dissolved in thionyl chloride (237 g, 2 mol), followed by stirring for 2 h at room temparature. The yellow solid was filtered, washed with cold petroleum ether (60–70 °C). Recrystallisation from water gave light-yellow crystals (115 g, 72%), melting point (m.p.) 146–147 °C.

5.2. 2-Methyl-5-hydroxy-4-pyrone (*2*)

Compound **1** (20 g, 0.12 mol) was suspended in water (500 mL). The temperature of the reaction mixture was raised to 50 °C. Zinc dust (16 g, 0.24 mol) was added and stirred at 70 °C for 0.5 h. Conc. HCl (13.6 g, 3 mol) was added dropwise followed by stirring for 4 h at 70–80 °C. The solution was filtered, poured into ice-water and extracted with dichloromethane, dried with Na_2SO_4 and evaporated to dryness. Recrystallisation of the resulting yellow solid from isopropanol provided compound **2** as light-yellow needles (10.2 g, 64%) m.p. 125–127 C.

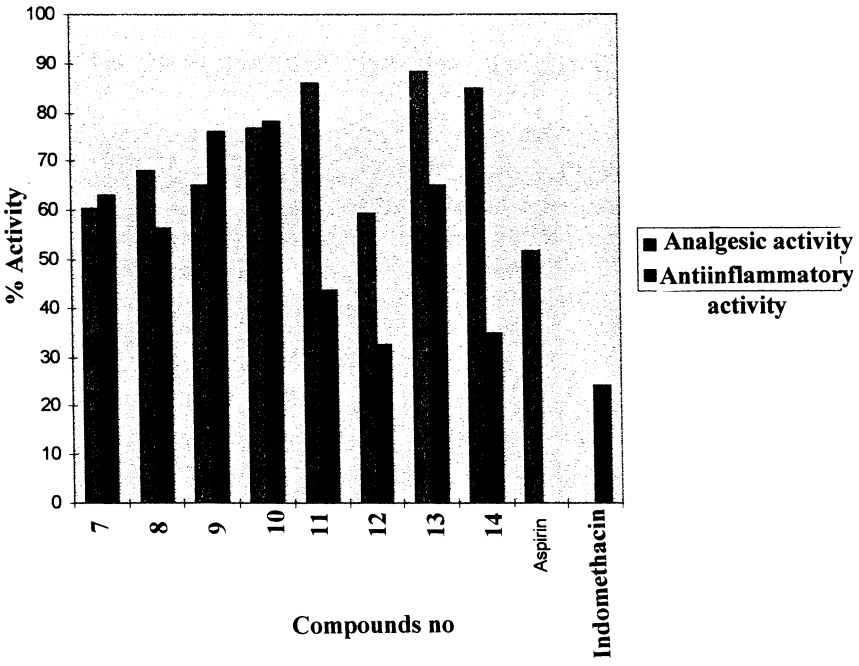


Fig. 3. Pharmacological activity of N-substituted-4(1H)-pyridinone·2HBr [**7**–**14**].

*5.3. 5-Benzyloxy-2-methyl-4-pyrone (**3**)*

Compound **2** (1 mol) was dissolved in methanol and the solution of NaOH (2 mol) in 10 mL water and benzyl chloride (2 mol) was added and heated under reflux for 8 h, then evaporated to dryness and extracted with dichloromethane, washed with 5% sodium hydroxide and water, dried with Na_2SO_4 and evaporated to dryness. Recrystallisation of the resulting tan solid from ether provided **3** as white needles (76%), m.p. 48 °C.

*5.4. General procedure for N-substituted-4(1H)-pyridinones (**7**–**10**)*

Compound **3** (1 mol) was dissolved in ethanol and primary amine was added. The mixture was heated under reflux for 40 h and evaporated to dryness, poured into the ice-water and extracted with chloroform. Organic phase was dried with Na_2SO_4 filtered and evaporated to dryness with vacuum to yield an orange oil.

*5.5. General procedure for N-substituted hydroxy-4(1H)-pyridinones (**11**–**14**)*

Compound **7a**–**14a** were dissolved in dry dichloromethane. BBr_3 in dichloromethane was added dropwise under nitrogen. The reaction was stirred for 3 h. The excess BBr_3 was destroyed at 15 °C by addition of cold methanol and stirred in an ice bath for 30 min. The mixture was concentrated to dryness in vacuum and the residue was several times dissolved in methanol and evaporated. Recrystallisation from ethanol–ether gave pure 4-pyridinone derivatives as yellow-white powder.

Acknowledgements

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